The utility of the lupus band test on sun-protected non-lesional skin for the diagnosis of systemic lupus erythematosus

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Abstract

Objective

The sensitivity and specificity of the lupus band test was evaluated, using three different criteria, on sun-protected non-lesional skin for the diagnosis of systemic lupus erythematosus (SLE). In addition, the sensitivity and specificity of the lupus band test was compared with those of other laboratory tests used in the diagnosis of SLE.

Methods

Sun-protected non-lesional skin biopsies from 65 patients (F 50; M 15; mean age 41 yrs.) with specific cutaneous manifestations of lupus erythematosus (LE) and from 18 patients with other dermatologic diseases (F 11, M 7; mean age 40 yrs.) were tested using the direct immunofluorescent technique.

Results

The sensitivity and specificity of the lupus band test was 10.5% and 97.8% respectively using the strict criterion of the presence of two different immunoreactants. The sensitivity and specificity were 52.6% and 69.5% respectively based on the presence of two different immunoreactants and were 78.9% and 47.8% based on the presence of only one immunoreactant. The highest sensitivity was found for ANA (100%). The specificity of all the laboratory abnormalities was particularly high, varying from 82.8% to 100%, except for ANA antibodies which showed a specificity of 65.2%.

Conclusions

A positive lupus band test on sun-protected non-lesional skin (even if showing the presence of only one immunoreactant at the dermo-epidermal junction) represents a useful and specific criterion for identifying patients with LE. However, this test is not useful in distinguishing between cutaneous lupus patients with systemic involvement and those without systemic involvement.

Key words

Lupus erythematosus, lupus band test, sun-protected non-lesional skin, antinuclear antibodies, immunoglobulins.

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Received on September 14, 1998; accepted in revised for on March 17, 1999.

Introduction

In 1963 Burnham *et al.*, using the direct immunofluorescence technique, demonstrated a band of localized immunoglobulins (Ig) at the dermo-epidermal junction in the affected skin of patients with discoid lupus erythematosus and systemic lupus erythematosus (SLE) (1). One year later, similar results were discovered in the seemingly normal skin of SLE patients (2) and the occurrence of complement components (C3 and C4) was found at the dermo-epidermal junction in skin lesions of patients with discoid LE and SLE (3). Since then, the term "lupus band test" had been employed to describe the deposition of Ig and complement at the dermo-epidermal junction in the cutaneous lesions of both discoid LE and SLE and in the normal appearing skin of SLE patients.

The prevalence of a positive lupus band test is much higher in lesional than in non-lesional skin, and in clinically normal sun-exposed skin than in clinically normal, non-sun-exposed skin (2-16). As the lupus band test can also be positive in 20% of sun-exposed skin specimens in normal healthy adults (17), it is clear that the test can be helpful in the diagnosis of LE only if performed on sun-protected non-lesional skin.

LE is a chronic, autoimmune inflammatory disease characterized by a spectrum of clinical forms; skin involvement occurs in 90% of all patients with LE. Patients may have cutaneous LE with or without systemic involvement (18)

There are many studies in which the lupus band test was performed on sun-protected non-lesional skin in SLE patients, but few of these stated whether cutaneous involvement was present at the time of the biopsy (19-22). We evaluated the utility of the lupus band test for the diagnosis of LE when performed on sun-protected non-lesional skin biopsies from 65 patients with specific cutaneous manifestations of LE (with or without systemic involvement) and from 18 patients with other dermatologic diseases. Moreover, to determine its utility for the SLE diagnosis, the sensitivity and specificity of the test on sun-protected non-lesional skin was determined and compared with those of other laboratory tests regularly used for the SLE diagnosis.

Materials and methods

Patients

Specimens of sun-protected non-lesional skin were taken from the buttocks (internal site) of 65 patients (50 females, 15 males; mean age 41 yrs.) with specific cutaneous manifestations of LE. According to Gilliam and Sontheimer's classification (23), the distinctive cutaneous subsets of LE examined were the following: chronic cutaneous LE (n = 43); subacute cutaneous LE (n = 13); and acute cutaneous LE (n = 9). Specimens of sun-protected non-lesional skin from 18 patients (F 11, M 7; mean age 40 yrs.) with other dermatologic diseases (polymorphous photodermatitis: n = 7; contact eczema: n = 4; pityriasis lichenoides: n = 1; seborrheic dermatitis: n = 4; chronic urticaria: n = 1 and androgenetic alopecia: n = 1) were used as controls.

On the basis of the American Rheumatism Association [now the American College of Rheumatology (ACR)] (24) criteria for the diagnosis of SLE, 19 out of 65 (localized discoid LE, n = 2; generalised discoid LE, n = 5; subacute cutaneous LE, n = 6; acute cutaneous LE, n = 6) could be included in the group of cutaneous LE patients with systemic involvement, while 46 out of 65 could be defined as having cutaneous LE without systemic involvement.

Twenty-one out of 43 patients (F 17, M 4; mean age 48 years) with chronic cutaneous LE had the localized type of discoid LE with lesions distributed over the face, external ear and scalp, while 19 out of 43 (F 10, M 9; mean age 39 yrs.) presented the generalised type with discoid lesions located not only on the face but also on the trunk (III° superior) and more rarely on the limbs. One out of 43 chronic cutaneous LE patients was included in the group because he was affected with lupus panniculitis (F, 42 yrs.) and 2 out of 43 chronic cutaneous LE patients were included for a chilblain lupus (both female, 25 and 39 years); 2 out of 21 patients with localized discoid LE (9.5%) met at least four of the ACR criteria for the diagnosis of SLE, while 5 out of 19 with generalised discoid LE (26.3%) could be considered LE-SLE patients.

The patients with lupus panniculitis and chilblain lupus did not meet a sufficient number of criteria for the diagnosis of

SLE. Thirteen patients showed a subacute form of cutaneous LE (F 12, M 1; mean age 44 years) and 6 out of 13 (46.1%) fulfilled at least four of the ACR criteria.
In the group of 9 patients with acute cutaneous LE (F 8, M 1; mean age 36 yrs), 6 out of 9 (66.6%) met at least four of the ACR criteria for SLE.

Table I. Sensitivity and specificity of the lupus band test for the diagnosis of SLE.

Criteria for lupus band test results	Sensitivity Cutaneous LE with systemic involvement	Specificity Cutaneous LE without systemic involvement	Specificity Control group
At least 1 immunoreactant	15/19 (78.9%)	22/46 (47.8%)	18/18 (100%)
At least 2 immunoreactants	10/19 (52.6%)	32/46 (69.5%)	18/18 (100%)
At least 2 different Ig	2/19 (10.5%)	45/46 (97.8%)	18/18 (100%)

Direct immunofluorescence procedure
After informed consent had been obtained, 4 mm punch biopsies were taken with 1% xylocaine local anaesthesia from each patient; the specimens were immediately frozen in liquid nitrogen and cut on a cryostat at -30°C into sections 4 μm thick. After a first washing with phosphate-buffered saline (PBS), the sections were incubated for 30 min in a wet chamber at room temperature with fluorescein-isothiocyanate (FITC) labelled mono-specific rabbit antisera to human IgG, IgM, IgA and C3 (Dako Glostrup, Denmark). These conjugates were used at a 1:20 dilution in PBS, except for the anti-IgG conjugate, which was used at a 1:50 dilution (25).
After incubation, the preparations were washed in PBS in a darkroom and then mounted in a medium made of glycerol in PBS, with sodium azide 0.1% added as a preservative (SIGMA Diagnostics, St. Louis, USA). The sections were examined with a fluorescence microscope (Leitz Orthoplan microscope, GMBH WETZZAR, West Germany). The evaluation of the fluorescent specimens was performed by two independent "blinded" observers.
In order to determine the sensitivity and specificity of the lupus band test for the diagnosis of SLE, we divided our lupus patients into two groups, those with and those without systemic involvement. Moreover, three different criteria were used for the definition of a positive lupus band test: (1) the lupus band test was considered as positive only if at least two different immunoglobulins were deposited at the dermo-epidermal junction at the same time; (2) the test was considered as positive if two different immunoreactants (an immunoglobulin and C3) were present at the dermo-epidermal junction; and (3) the test was considered as positive if at least one immunoreactant (any Ig or C3) was deposited at the dermo-epidermal junction.
Sensitivity and specificity were determined using standard methods, summarised as follows:

Sensitivity (%) = True positives/ True positives + False negatives.
Specificity (%) = True negatives/ True negatives + False positives.
True positives = lupus patients with cutaneous and systemic involvement positive for the examined test
False negatives = lupus patients with cutaneous and systemic involvement negative for the examined test
True negatives = patients with cutaneous but not systemic involvement negative for the examined test
False positives = patients with cutaneous but not systemic involvement positive for the examined test

The sensitivity and specificity of the other tests (tests for anemia, leukopenia, thrombocytopenia, serum gammaglobulin levels, circulating antinuclear (ANA) and anti-ds-DNA antibodies, circulating immune complexes (IgG binding C1q and C3) and complementemia (low serum levels of C3 and C4) were determined using the same standard method. Antinuclear and anti-ds-DNA antibodies were detected using indirect immunofluorescence with rat liver sections (26) and *Crithidia luciliae* smears, respectively (27). The level of circulating immune complexes (IgG binding C1q and C3) was determined by an enzyme immunoassay (28). The red blood cell, white blood cell and platelet counts were performed using an electronic meter (Technicon). The C3 and C4 assay was carried out using a simple radial immunodiffusion technique (29).

Results

The sensitivity and specificity of the lupus band test on sun-protected non-lesional skin (Table I) depended upon the criteria used for their evaluation. Using the strictest criterion (at least two different immunoglobulins at the dermo-epidermal junction), the sensitivity of the lupus band test was 10.5%. Using a less rigorous criterion (two different immunoreactants, an immunoglobulin and C3, at the dermo-epidermal junction) the sensitivity was 52.6%, while with the third criterion (at least one immunoglobulin or C3) the sensitivity was 78.9%. On the other hand the specificity was 97.8% using the most rigorous criterion, 69.5% using the intermediate one, and 47.8% using the least rigorous one. The specificity of the lupus band test in the patients with dermatologic diseases other than LE was very high, being 100% using any one of the 3 different criteria.
As regards the sensitivity and specificity of the other investigations for the diagnosis of SLE (Table II), the search for ANA was found to be the most sensitive test (100%). The tests for low serum levels of C4 and high serum levels of circulating immunocomplexes (IgG-binding C3) usually used to estimate disease activity revealed a sensitivity of 52.6% for the former and 50% for the latter. The search for low serum levels of C3 and high serum levels of other circulating immunocomplexes (IgG-binding C1q) instead showed a low sensitivity, the former being 31.5% and the latter being 12.5%. Leukopenia showed only a slight sensitivity, 42.1%, as did the search for high serum levels of gammaglobulins, 47.3%. The search for anti DNA-ds antibodies, anemia and thrombocytopenia provided results with a low sensitivity, 12.5%, 10.5% and 15.7%, respectively. In contrast, the specificity of these laboratory tests was particularly high, varying from 82.8% to 100% except for the antinuclear antibody results, that showed a specificity of 65.2%.

Table II. Sensitivity and specificity of serological investigations for the diagnosis of SLE.

	Sensitivity Cutaneous + systemic lupus	Specificity Cutaneous but not systemic lupus
↑ gammaglobulins	9/19 (47.3%)	38/41 (92.6%)
↓ C3	6/19 (31.5%)	33/36 (91.6%)
↓ C4	10/19 (52.6%)	29/35 (82.8%)
↑ IgG complexes binding C1q	2/16 (12.5%)	33/33 (100%)
↑ IgG complexes binding C3	8/16 (50%)	28/33 (84.8%)
ANA	19/19 (100%)	30/46 (65.2%)
Anti-ds-DNA	2/16 (12.5%)	13/14 (92.8%)
↓ red corpuscles	2/19 (10.5%)	46/46 (100%)
↓ white corpuscles	8/19 (42.1%)	45/46 (97.8%)
↓ platelets	3/19 (15.7%)	43/46 (93.4%)

Discussion

In this study on the validity of the lupus band test for the diagnosis of SLE, we chose to study a sun-protected non-lesional site, specifically the buttock region because this is generally sun-protected in spite of different latitudes and social customs. We decided to avoid clinically normal sun-exposed skin because of reports of 20% positive results obtained from biopsies of sun-exposed (but not sun-protected) skin of normal healthy adults (17).

To date studies of the lupus band test have been conducted on sun-protected non-lesional skin in SLE patients, but few authors have examined these results in relation to the cutaneous manifestations of LE (19-22). The aim of this study was to compare lupus band test results for sun-protected non-lesional skin in cutaneous LE patients with and without systemic involvement, in order to determine if any meaningful differences could be found.

Previous studies have shown a wide variability in the results of the lupus band test on sun-protected non-lesional skin (19-22, 30-43) (Table III). This is probably due to the different criteria used for the evaluation of positive results and to different regions from which skin was taken for the biopsy. Therefore, in this study we used three different criteria to evaluate the lupus band test in order to identify the most suitable criterion for SLE diagnosis.

The sensitivity of the lupus band test clearly varied as a function of the criteria used for the evaluation of the test. It was therefore difficult to choose a standard criterion for the definition of a positive lupus band test on sun-protected non-lesional skin in order to obtain an additional element, besides the ARA criteria, for the diagnosis of SLE.

The most rigorous criterion lowered the false-positive result rate so that cutaneous LE patients without systemic involvement could be excluded. At the same time this criterion had a very low sensitivity (10.5%) and was associated with a high rate of false-negative results. With the less rigorous criterion the lupus band test showed good sensitivity (78.9%) but a high rate of false-positive results, the specificity being 47.8%.

So far, no study has been performed to determine the sensitivity and specificity of the lupus band test on sun-protected non-lesional skin. In a study by George *et al*. (44), biopsies were taken from lesional skin or sun-exposed areas of the upper limbs, while in a study by Scarpa *et al.* (45) clinically normal, sun-exposed skin was tested. It is important to point out that in George *et al.*'s study the controls were chosen from a group of patients who had lesions, but which histopathology, laboratory tests and follow-up showed were not due to discoid or systemic LE. In Scarpa's study the controls were patients with other rheumatologic diseases. The originality of our study consists of the choice of CLE-non SLE patients as controls, in addition to patients with other dermatologic diseases.

When the sensitivity and specificity of the lupus band test was compared to that of other laboratory tests used for the diagnosis of SLE, we found that the ANA assay was the test with the highest sensitivity (100%) (Table II). Therefore, the presence of these antibodies can be considered an essential condition for the diagnosis of SLE even if it should be pointed out that ANA had a low specificity (65.2%) in comparison with the other laboratory tests. It is very interesting to note the different "behaviour" of C3 and C4 which could be observed because the decrease in C4 was more sensitive (52.6%) than that in C3 (31.5%) even if the serum decrease in these factors is linked to the same mechanism (consumption by immune complexes).

In conclusion, a positive lupus band test (even if based on the presence of only one immunoreactant at the dermo-epidermal junction) when performed on sun-protected non-lesional skin constitutes a useful and specific criterion for identifying patients with LE. This test cannot, however, distinguish between cutaneous lupus patients with and without systemic involvement. In our opinion, only the follow-up of CLE-non SLE patients with a positive lupus band test on sun-protected non-lesional skin will confirm the prognostic importance of this test. This last observation implies the need to classify patients with LE associated with cutaneous involvement not only by the ARA criteria but also by the European Academy of Dermatology and Venerology (EADV) study factors (46). According to these additional criteria suggested by EADV, we stress the importance, not only of discoid lesions or malar rash, but also of papulosquamous or annular lesions in the examination of patients with CLE.

Although added EADV study factors include direct IF of normal exposed skin from the wrist and of lesional skin, preferably not the face, we believe that the best choice to perform the test is on sun-protected non-lesional skin.

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Table III. Composition of lupus band test on sun-protected non-lesional skin in different studies of lupus erythematosus patients.

Authors	Variety	Site of biopsy	IgG	IgA	IgM	C_3
Jordon *et al.*, 1974 (ref. 34)	SLE	not specified	6/11* (54.5%)†	2/8 (25%)	11/11 (100%)	8/11 (72.7%)
Gilliam, 1975 (ref. 35)	SLE	not specified	37/47 (78.7%)	8/47 (17%)	37/47 (78.7%)	26/47 (55.3%)
Dantzig *et al.*, 1975 (ref. 40)	SLE	lower back	11/24 (45.8%)	0	11/24 (45.8%)	4/24 (16.6%)
Noel *et al.*,1978 (ref. 36)	SLE	flexor area forearm	8/17 (47%)	1/17 (5.8%)	7/17 (41.1%)	4/17 (23.5%)
O'Loughlin *et al.*, 1978 (ref. 19)	Localized discoid LE Generalized discoid LE SLE	not specified	0 0 13/46 (28%)	0 0 2/20 (10%)	0 5/28 (18%) 42/46 (91%)	0 3/29 (10%) 16/45 (36%)
Permin *et al.*,1979 (ref. 37)	SLE	flexor area upper arm	11/39 (28%)	3/39 (8%)	24/39 (62%)	14/39 (36%)
Ahmed *et al.*, 1979 (ref. 30)	SLE	buttocks	7/7 (100%)	1/7 (14.2%)	2/7 (28.5%)	0
Sontheimer *et al.*, 1979 (ref. 38)	SLE	flexor area forearm	22/30(73.3%)	10/30(33.3%)	29/30(96.6%)	not studied
Sontheimer *et al.*, 1979 (ref. 42)	Subacute cutaneous LE	flexor area forearm	5/26 (19.2%)	3/26 (11.5%)	6/26 (23%)	not studied
Halberg *et al.*, 1982 (ref. 39)	SLE	buttock	17/58 (29.3%)	16/58 (27.5%)	47/58 (81%)	13/58 (22.4%)
Jacobs *et al.*, 1983 (ref. 32)	SLE	buttock (internal site) buttock (medial site) buttock (lateral site)	4/11 (36.3%) 5/9 (55.5%) 4/9 (44.4%)	1/11 (9%) 1/9 (11.1%) 0	9/11 (81.8%) 6/9 (66.6%) 8/9 (88.8%)	1/11 (9%) 0 1/9 (11.1%)
Davis *et al.*, 1984 (ref. 31)	SLE	flexor area forearm	19/29 (65.5%)	not studied	10/29 (34.4%)	not studied
Smith *et al.*, 1984 (ref. 43)	-SLE	deltoid area	50/102 (49%)	25/102 (25%)	56/102 (55%)	49/102 (48%)
Gabrielli *et al.*, 1985 (ref. 33)	SLE	flexor area forearm	15/23 (65.2%)	5/23 (21.7%)	14/23 (60.8%)	2/23 (8.6%)
Oxholm *et al.*, 1986 (ref. 41)	SLE	buttock	2/13 (15%)	0	0	0
Benoldi *et al.*, 1987 (ref. 22)	SLE with nephritis/vasculitis SLE without nephritis/vasculitis Generalised discoid LE Localized discoid LE	flexor area forearm	5/13 (38.4%) 2/17 (11.7%) 0 0	2/13 (15.3%) 0 0 0	9/13 (69.2%) 11/17(64.7%) 7/19 (36.8%) 1/12 (8.3%)	7/13 (53.8%) 5/17 (29.4%) 2/19 (10.5%) 0
Gruschwitz *et al.*, 1988 (ref. 21)	Discoid LE	inside upper arm	0	0	6/10 (60%)	0
Burge *et al.*, 1989 (ref. 20)	SLE Discoid LE	forearm	1/6 (16.6%) 0	1/6 (16.6%) 0	3/6 (50%) 1/32 (3.1%)	1/6 (16.6%) 0

*Number of patients studied; †percentage of positive results.
Smith *et al.*, 1984 (ref. 43): C4 17/102 (17%); C1q 45/102 (44%); Properdin 21/102 (21%)

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